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► To cite this version:

Christophe Pichon, Brice Felden. Proteins that interact with bacterial small RNA regulators.. FEMS Microbiology Reviews, 2007, 31 (5), pp.614-25. 10.1111/j.1574-6976.2007.00079.x . inserm-00712150

HAL Id: inserm-00712150

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Submitted on 26 Jun 2012

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REVIEW ARTICLE

Proteins that interact with bacterial small RNA regulators

Christophe Pichon & Brice Felden

Inserm U835, Upres JE2311, Biochimie Pharmaceutique, Université de Rennes 1, Rennes, France

Correspondence: Brice Felden, Inserm U835, Upres JE2311, Biochimie Pharmaceutique, Université de Rennes 1, Rennes, France. Tel.: +33 2 23 23 48 51; fax: +33 2 23 23 44 56; e-mail: bfelden@univ-rennes1.fr

Present address: Christophe Pichon, Institut Pasteur, Unité 'Pathogénie Bactérienne des Muqueuses', Paris, France.

Received 25 March 2007; revised 12 June 2007; accepted 14 June 2007.
First published online 26 July 2007.

DOI:10.1111/j.1574-6976.2007.00079.x

Editor: Cecilia Arraiano

Keywords

RNA-binding protein; RNA chaperone; small RNAs; noncoding RNAs; regulatory RNAs; sequestering RNAs.

Introduction

Over the last decade, we have seen the structures of essentially all the major RNA-binding protein families solved, which in turn has helped us elucidate how RNA recognition proceeds. The ribosome atomic structures have allowed the integration of this knowledge into principles for the assembly of complex ribonucleoproteins. 'Protein–RNA' interactions usually occur through induced fit, resulting in conformational changes of one or both ligands, and thus in general increasing the structural order of the RNA. RNA structure consists of double helical elements that are connected by bulges, internal loops or junctions and assembled by tertiary (long-distance) interactions, as well as by the interacting proteins. RNA helices can change orientation upon protein binding. RNAs undergo structural rearrangements, from fast localized motions of the residues involved in protein recognition to slower reorientations of helical domains due to the flexibility of nucleotides at hinge positions. These movements are modified upon binding of protein ligands. When a protein binds RNA, its conforma-

Abstract

Small regulatory RNAs have been identified in a wide range of organisms, where they modify mRNA stability, translation or protein function. Small RNA regulators (sRNAs) either pair with mRNA targets or modify protein activities. Here we discuss current knowledge of the various proteins that interact with RNA regulators and review the physiologic implications of sRNA–protein complexes in DNA, RNA and protein metabolism, as well as in RNA and protein quality control in prokaryotes. Proteins that interact with the sRNAs can possess catalytic activity, induce conformational changes of the sRNA, or be sequestered by the sRNA to prevent the action of the protein.

tion is usually altered or stabilized. These structural changes are essential for the ability of RNAs to modulate gene expression. 'Protein–RNA' recognition is an essential process during gene expression. Also, various posttranscriptional and translational regulatory events depend on specific interactions between proteins and small regulatory RNA (sRNAs).

In prokaryotes, 'small' RNAs, also referred to as 'noncoding' or 'regulatory' RNAs (sRNAs), function as gene expression regulators (Storz *et al.*, 2005). Their size ranges from 50 to 550 nucleotides. So far, we have learned most about the sRNAs expressed in *Escherichia coli*, but more are being identified in other species (Pichon & Felden, 2005; Mandin *et al.*, 2007). Most sRNAs that have been ascribed a biological function interact with specific protein(s) (Table 1). Regulatory RNAs possess intrinsic dynamic structures that are exploited by proteins to trigger specific interactions, eliciting a biological response. sRNAs can either act through base-pairing interactions with target RNAs, or form functional ribonucleoprotein complexes. The sRNAs that pair with target RNAs can be temporarily assisted by specific

Table 1. The proteins that interact with bacterial sRNAs

Proteins	Classes	Associated sRNA(s)	Functions
FinO	RNA chaperone	FinP	Conjugation control
Rom	RNA chaperone*	RNAI	Regulation of plasmid copy numbers
σ^{70} -RNA polymerase	Sequestered	6S RNA	Regulation of σ factor utilization
C5	Part of an RNA enzyme	M1 RNA ^{†,‡}	5'-End maturation of sRNAs
RNase III	Enzyme	‡	sRNA maturation, stability and decay
RNase E	Enzyme	‡	Translational control sRNA maturation, stability and decay
RNase H	Enzyme	RNAI	Translational control DNA replication initiation
RNase T (RNase PH)	Enzyme	tmRNA, 4.5S RNA, 6S RNA, others?	3'-End maturation of sRNAs
Hfq	RNA chaperone	‡	RNA stability and decay Translational control
Alanyl-tRNA synthetase	Enzyme	tmRNA	Aminoacylation of tmRNA with alanine
EF-Tu	Transporter	tmRNA	Transport Protection of the aminoacyl-ester bond
SmpB	RNA chaperone*	tmRNA	<i>Trans</i> -translation
S1	RNA scaffolding	DsrA, tmRNA	Translational control Translation, <i>trans</i> -translation
Ffh	RNA scaffolding	4.5S RNA	Protein trafficking
StpA	RNA chaperone	MicF	sRNA quality control
Ro	RNA chaperone*	Y1–Y4	sRNA quality control?
CsrA	Sequestered	Csr B, Csr C, Csr D	Carbon storage regulation

*The RNA chaperone functions have been only inferred, and additional roles are expected.

[†]The M1 RNA has the catalytic activity.

[‡]Many sRNAs interact with these proteins or these ribonucleoproteins.

proteins before, during or after the 'sRNA–target RNA(s)' interaction(s). These proteins can facilitate the recognition between the two interacting RNAs (RNA chaperones, proteins that promote folding of RNAs by loosening their structures) or they can induce the specific hydrolysis of the target RNA(s) and also the degradation of the sRNA, to ensure that the regulator is rapidly turned off once it has exerted its regulatory function (RNases, e.g. RNases III and E). The possible consequences of the pairing (Fig. 1a) are translational inhibition [e.g. spot 42 RNA (Moller *et al.*, 2002)] or stimulation [e.g. DsrA RNA (Sledjeski *et al.*, 2001)], and mRNA stabilization [e.g. GadY RNA (Opdyke *et al.*, 2004)] or degradation [e.g. RyhB RNA (Massé *et al.*, 2003)]. Within ribonucleoprotein complexes (Fig. 1b–d), the sRNA forms an intricate scaffold that contains specific binding sites for the associated protein(s). In these complexes, the free sRNA is either inactive [e.g. the 4.5S RNA from the signal recognition particle, transfer-messenger (tm)RNA in the absence of the small protein B (SmpB)] or sustains some activity (the RNase P RNA in the absence of protein C5; however, the protein is required for activity *in vivo*). Therefore, for many sRNAs, the associated proteins are essential for activity.

This review covers the functional importance of proteins in 'sRNA-mediated' regulation of gene expression in bacteria, summarizes their implications for major cellular mechanisms, and provides a perspective on future research on

sRNA-binding proteins. The focus is on the physiologic processes in which the proteins that bind sRNAs are involved. Although 'sRNA–protein' complexes have been identified in many genera of bacteria, they have been most extensively studied for *E. coli*. Where appropriate, data obtained from other organisms are provided. Proteins that interact with riboswitches and other regulatory mRNA domains responsible for modulation of gene expression are beyond the scope of this review.

DNA metabolism

DNA transfer regulation

Bacteria can exchange DNA through a process called conjugation (Frost *et al.*, 1994). The *E. coli* F plasmid contains genes necessary for conjugation. Among these, *traJ* expresses a transcriptional activator that is under the negative control of two *fin* genes (fertility inhibition system), the *cis*-encoded 79-nucleotide *finP* sRNA, and the 186 amino acid *finO* protein. *FinP* is an antisense RNA that is partially complementary to the 5'-untranslated region (5'-UTR) of *traJ* mRNA. Inhibition of conjugation leads to the transcription of the *FinP* sRNA that binds the *traJ* mRNA Shine–Dalgarno sequence. Consequently, *traJ* is not translated, and degradation of the sRNA–mRNA complex is initiated by RNase III (Jerome *et al.*, 1999).

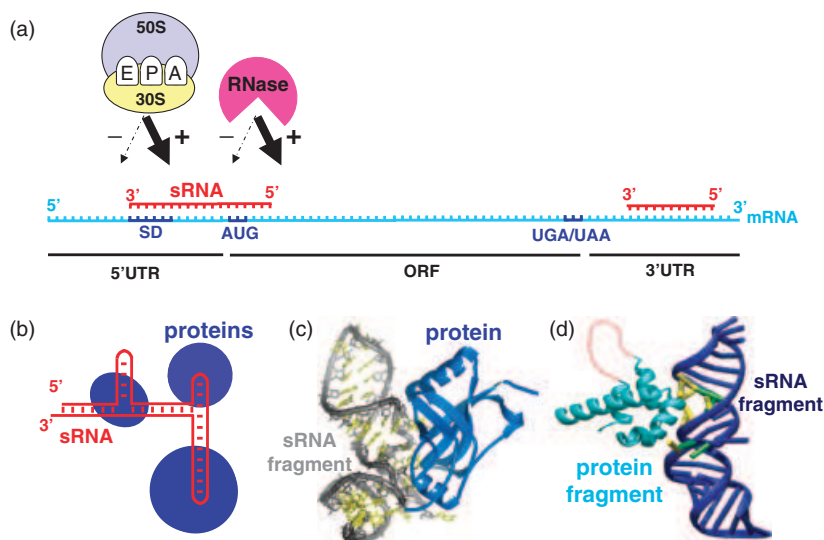


Fig. 1. Bacterial sRNAs act through base-pairing interactions with target RNAs or form persistent functional ribonucleoprotein complexes. (a) sRNAs (red) can repress or enhance initiation of protein synthesis by blocking (–) or promoting (+) ribosome (blue-yellow) binding to the mRNAs (light blue) by pairing at or around the Shine–Dalgarno/initiation codon (dark blue). sRNAs can also destabilize (–) or prevent degradation (+) of mRNA targets by increasing or decreasing accessibility to various RNases (purple) by triggering specific pairings with RNA targets. Bacterial mRNAs usually contain 5′/3′-UTRs flanking the ORF starting at an AUG initiation codon (dark blue) and ending at UGA or UAA termination codons (dark blue). Bacterial sRNAs pair with either the 5′-UTR/AUG codon (Massé *et al.*, 2003) or the 3′-UTR (Opdyke *et al.*, 2004). In *Escherichia coli*, the sRNAs that pair to mRNA targets bind transiently to various proteins, including the hexameric Hfq protein, as do the target mRNAs. (b) Prokaryotic sRNA transcripts are usually highly folded (red) and form specific recognition sites for dedicated proteins (blue). Examples of two atomic structures of functional ribonucleoprotein complexes are presented: (c) the tRNA domain of tmRNA in complex with smpB involved in *trans*-translation (Gutmann *et al.*, 2003); and (d) the structure of the conserved domain of the *Escherichia coli* 4.5S sRNA (dark blue ribose-phosphate backbone) bound to the Ffh protein M domain (Doudna & Batey, 2004) (light blue protein backbone ribbon) of the SRP involved in cotranslational protein trafficking. Nucleotides in the 4.5S RNA that are highly (green) or universally (yellow) conserved are highlighted.

The mRNA–sRNA interaction is triggered by the presence of two ‘U-turn’ motifs in both stem-loops of FinP (Franch *et al.*, 1999). *In vivo*, the interaction is stabilized by FinO, which prevents RNase E degradation of the two RNAs (Ghetu *et al.*, 1999). Monomeric FinO binds one stem-loop with flanking nucleotides of FinP. The three-dimensional structure of FinO lacking its 25 first N-terminal residues has been solved (Ghetu *et al.*, 2003), and comprises two β -sheets and six α -helices, two of which form an RNA-binding site and recognize the global conformation of the two RNAs (Ghetu *et al.*, 1999). Although the 25 positively charged N-terminal residues of FinO do not participate in the overall protein structure, there is a 10-fold reduction of RNA duplex formation in their absence, probably preventing electrostatic repulsion between the two RNAs. It has been proposed that FinO promotes and stabilizes duplex formation without the need for ATP, suggesting that the protein is an RNA chaperone (Arthur *et al.*, 2003).

Plasmid replication control

Plasmids replicate independently of chromosomal DNA to maintain adequate copy number in bacterial cells. The

ColE1 plasmid controls its replication via the 108-nucleotide RNAI antisense sequence (Eguchi *et al.*, 1991). In the absence of RNAI, RNA polymerase transcribes a noncoding RNAII transcript of a promoter located 555 nucleotides upstream of the replication origin. When the transcription machinery reaches the origin, the 3′-end of RNAII is cleaved by RNase H and forms a stable RNA–DNA hybrid that is used by DNA polymerase I to trigger plasmid replication. When RNAI is expressed, it binds the nascent RNAII during transcription, inhibiting primer formation and replication. RNAI contains three stem-loops with U-turn motifs that form a kissing complex with three complementary stem-loops of RNAII (Franch *et al.*, 1999). A protein stabilizes this RNA kissing complex and protects it against degradation (Binnie *et al.*, 1999). Complex formation between RNAI and RNAII is stabilized by the 63 amino acid protein Rom (RNA one modulator, also named repressor of primer or Rop) encoded by the ColE1 plasmid. Rom is active as a dimer that binds to and stabilizes the RNAI–RNAII kissing complex, protecting it from RNase degradation. Structural studies show that Rom has a central cavity where the two RNAs bind (Jang *et al.*, 2006).

RNA metabolism

Transcription

Gene expression is tightly regulated at the transcriptional level, especially during the initiation step. Bacterial RNA polymerase (RNAP) synthesizes RNA from DNA templates. The RNAP core enzyme contains four polypeptide chains ($\alpha_2\beta\beta'$), and additional σ subunits help the core enzyme recognize promoter sequences. In bacteria, the level of each of the σ factors is tightly regulated to ensure that the appropriate genes are expressed in response to environmental changes. In *E. coli*, nutrient limitation during the stationary phase of growth reduces the expression of a large set of genes with promoter sequences that are recognized by the σ^{70} factor. Conversely, the transcription of genes under the control of the σ^S factor increases. Various regulators control the σ^{70} to σ^S transition, including the 184 nucleotide 6S sRNA (Fig. 2a). This RNA has a compact secondary structure that mimics an open DNA promoter and inhibits σ^{70} -driven transcription initiation by binding up to 75% of the ' σ^{70} -RNAP' complexes that are locked into an inactive state, favoring σ^S gene-dependent transcription (Wassarman & Storz, 2000). The ' σ^{70} -RNAP-6S RNA' complex initiates transcription from 6S RNA template, synthesizing 14 to 20 nucleotide-long RNA (pRNA) that destabilizes the 'RNA-protein' complex, freeing the ' σ^{70} -RNAP' complex. The pRNA 6S RNA is released and its cellular level decreases rapidly, probably due to RNase digestion (Wassarman & Saecker, 2006). The 6S RNA has been detected in many

species, suggesting that the 6S RNA-RNAP interaction was maintained during bacterial evolution for efficient gene promoter switches during growth (Barrick *et al.*, 2005; Pichon & Felden, 2005). The structure of the ' σ^{70} -core RNAP-6S RNA' complex is not known, and its comparison with a canonical 'RNAP-DNA promoter' complex would give important clues as to how sRNA regulation proceeds at the molecular level.

Maturation and decay

Once a primary sRNA transcript has been synthesized, it is usually processed by various endoribonucleases and exoribonucleases (RNases), producing a functional molecule that, in turn, will be ultimately degraded (Fig. 2b). The boundary between RNA maturation and decay is rather ambiguous (Nicholson, 1999). Maturation and decay were initially investigated with rRNAs and transfer (t)RNAs (Deutscher, 2006). Specific RNases are responsible for the maturation of several sRNAs expressed in *E. coli* and probably in other species as well. Examples of such sRNAs are the RNase P RNA, the 4.5S and 6S RNAs, tmRNAs and most likely other sRNAs (Deutscher, 2006). Prokaryotic RNases have a variety of structures, mechanisms and functions, including RNA maturation, RNA decay and a direct implication in sRNA-mediated gene regulation. Several enzymes, such as RNase P, III, E, G, H, T and PH, are involved in both mRNA and sRNA maturation, function and decay.

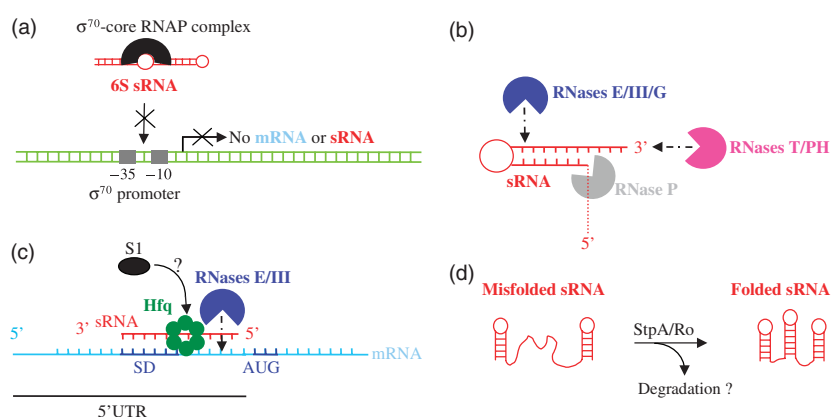


Fig. 2. The proteins that interact with bacterial sRNAs during RNA metabolism. (a) RNA synthesis by the ' σ^{70} -core RNA polymerase' complex (black). 6S sRNA (red) sequesters the polymerase complex during nutrient limitation (stationary phase of growth), requiring the expression of genes controlled by an alternative σ factor (σ^S). (b) sRNA maturation mediated by various RNases. Endoribonucleases E, III, G (blue) and P (grey) cleave RNA internally and contribute to the formation of mature and functional sRNAs. Exoribonucleases T and PH (purple) perform 3'-end maturation of many sRNAs. (c) Many bacterial antisense sRNAs pair with the 5'-UTRs of target mRNAs (light blue), some with the assistance of protein Hfq (green) and possibly ribosomal protein S1 (black). If the sRNA-mediated regulation results in mRNA translation inhibition, the targeted mRNA is cleaved by RNases E or III, triggering mRNA decay, probably with the help of the RNA degradosome (Massé *et al.*, 2003; Huntzinger *et al.*, 2005). The RNases can also cleave and trigger the degradation of the sRNA regulator. (d) sRNAs (red) can function thanks to intact three-dimensional structures. When the conformation of an sRNA is locked into a kinetic trap, specific proteins can bind to the sRNA, unfold and allow the sRNA to refold into a functional conformation.

RNAse P is a ubiquitous endonucleolytic enzyme that catalyzes the maturation of the 5'-end of tRNA (Kazantsev & Pace, 2006). In bacteria, this RNAse consists of a protein (C5) and RNA (M1). In bacteria and some archaea, the RNA component of RNAse P can catalyze tRNA maturation *in vitro* in the absence of proteins (ribozyme), but the protein is required for function *in vivo* (Buck *et al.*, 2005). The identified protein-binding site on bacterial RNAse P RNA is located adjacent to the proposed chemically active site, and the current belief is that the protein has no direct role in catalysis (Harris & Christian, 2003). RNAse P has additional RNA substrates, including the 5'-ends of several pre-sRNAs, such as tmRNA, 4.5S RNA and probably others, indicating its importance in sRNA maturation (Peck-Miller & Altman, 1991; Komine *et al.*, 1994), in addition to its role in the 5'-maturation of precursor tRNAs and in rRNA maturation.

Studies on rRNA and tRNA maturation have led to the discovery of a wealth of RNAse activities, including RNAse III. RNAse III is a ubiquitous enzyme in bacteria, and homologs have been identified in eukaryotes (MacRae & Doudna, 2007). RNAse III cleaves 20 nucleotide double-stranded RNAs, independently of the nucleotide sequence. In bacteria, RNAse III has been implicated in the maturation of some sRNAs (Argaman *et al.*, 2001), and in the antisense sRNA-induced decay of some mRNAs (Huntzinger *et al.*, 2005), and alters the decay pathway of RNAI, the antisense inhibitor of ColE1 replication (Binnie *et al.*, 1999).

Another endoribonuclease, RNAse E, which is not ubiquitous in bacteria, is also involved in bacterial mRNA translation and decay (Carpousis, 2002; Folichon *et al.*, 2003), in the coupled degradation of mRNA-sRNA duplexes (Massé *et al.*, 2003; Morita *et al.*, 2005), and initiates CopA and RNAI decay (Soderbom & Wagner, 1998; Binnie *et al.*, 1999). The fate and decay of the sRNAs is much less understood than that of the mRNAs, but recent data suggest that Hfq, RNAse E and some other components of the RNA degradosome could play important roles in RyhB decay and probably also for other sRNAs (Massé *et al.*, 2003). RNAse E has not been identified in *Staphylococcus aureus* and in some other gram-positive species (Condon & Putzer, 2002), suggesting that sRNA maturation and sRNA-mRNA decay are caused by RNAse(s) yet to be discovered. However, functional homologs of RNAse E that retain such features as substrate recognition and cleavage specificity, RNases J1 and J2, were recently identified in *Bacillus subtilis* (Even *et al.*, 2005).

Like RNAse III, RNases E and G participate in sRNA maturation, function and decay in *E. coli*. RNases E and G exhibit sequence similarity and functional properties. They also cleave sRNAs at specific sites, suggesting binding site specificity (Carpousis, 2002; Callaghan *et al.*, 2005). RNases E and G are involved in tmRNA (Lin-Chao *et al.*, 1999) and 6S RNA (Kim & Lee, 2004) maturation and function. This

probably holds true for other sRNAs (Vogel *et al.*, 2003). Antisense sRNAs regulate message stability and/or translation of one or several target mRNAs. Bacterial sRNAs such as RNAI (Kaberdin *et al.*, 1996), SgrS or RyhB (Morita *et al.*, 2005) trigger mRNA decay by inducing their cleavage by RNAse E, most likely in response to the formation of the mRNA-sRNA duplex. In *E. coli*, this process involves the Hfq protein (see below). In *E. coli*, sRNA-mediated mRNA degradation involving RNases E and III is linked to other components of the RNA degradosome (Morita *et al.*, 2005). In the absence of their target mRNAs, the decay of the regulatory sRNA is mainly initiated by RNAse E and potentially also by RNAse III. In the case of CopA sRNA (Soderbom & Wagner, 1998; Carpousis, 2002), RNAse II and poly(A) polymerase I complete its degradation at the nucleotide level. This degradation pathway is far from being elucidated for each sRNA, but poly(A) tailing of sRNAs by poly(A) polymerase I is a redundant observation (Argaman *et al.*, 2001; Dreyfus & Régner, 2002).

RNAse H is another endoribonuclease that binds to and cleaves RNA within RNA-DNA duplexes, and is essential during ColE1 plasmid replication. In the absence of sRNA RNAI, RNAse H cleaves RNAI to produce a functional RNA primer, allowing DNA replication initiation by DNA polymerase I (see above). Similarly, RNAse H could be involved in transcriptional control by other antisense sRNAs that target mRNA-DNA heteroduplexes (Massé & Gottesman, 2002).

RNAse cleavage at 3'-ends of RNAs was initially observed for tRNAs and 5S RNA from *E. coli* (Li & Deutscher, 1995). Among the exoribonucleases expressed in *E. coli*, RNases T and PH are the two most effective enzymes for removing 3'-nucleotides from various sRNAs (Li *et al.*, 1998). Several sRNAs, including the M1 RNA, tmRNA, 6S RNA and 4.5S RNA, undergo exoribonucleolytic trimming of their 3'-ends, a process that contributes to their functionality. In contrast to other exoribonucleases, RNAse T removes unpaired nucleotides from the 3'-end to the 5'-end of sRNAs and may not be implicated in sRNA decay (Nicholson, 1999). The 5'-ends and the 3'-ends of three well-characterized sRNAs (4.5S RNA, 6S RNA and M1 RNA), which are recognized and processed by RNases T and PH, pair and form stable helices that prevent the progression of the enzymes into the sRNA structures. The sequence of the 3'-ends of these sRNAs is NCC-3', where N can be any of the four bases. *In vitro* experiments indicate that a synthetic RNA helix followed by an NCC-3' single strand reduces or prevents RNAse T activity (Zuo *et al.*, 2002). In the absence of these exoribonucleases, the maturation of 4.5S and M1 RNAs cannot proceed, but these two sRNAs are still functional. For tmRNA, other RNases can replace the missing ones to form a mature RNA (Lin-Chao *et al.*, 1999). The exoribonuclease RNAse R is also involved in the maturation

of tmRNA (Cairrão *et al.*, 2003), and degrades nonstop mRNA selectively in an SmpB–tmRNA-dependent manner (Richards *et al.*, 2006).

Trans-acting helpers

Host factor 1 (HF1 or Hfq) was initially identified as a protein required for bacteriophage Q β RNA replication in *E. coli* (Franze de Fernandez *et al.*, 1972). In *E. coli*, the deletion of *hfq* induces pleiotropic effects on growth and survival during various forms of stress (Tsui *et al.*, 1994) (e.g. osmotic shock, oxidative damage). These effects could be explained by the direct and indirect roles of Hfq on posttranscriptional regulation by targeting sRNAs and mRNAs (Vecerek *et al.*, 2003). Hfq controls the translation (Muffler *et al.*, 1996; Tsui *et al.*, 1997) and decay (Folichon *et al.*, 2003) of some mRNAs in an 'sRNA-independent' manner. Several antisense sRNAs, however, need Hfq to interact with target mRNA(s) that, in turn, modify mRNA translation and/or stability. Hfq also stabilizes the interacting sRNAs *in vivo* (Sledjeski *et al.*, 2001; Antal *et al.*, 2005). In this review, the focus is on the relationships of Hfq with sRNAs.

By facilitating the interaction between some sRNAs and their associated mRNA targets, the protein participates in the positive or negative regulation of translation initiation of these mRNAs (Massé *et al.*, 2003; Grieshaber *et al.*, 2006). Hfq can also induce mRNA stabilization with the help of an sRNA (Opdyke *et al.*, 2004). Several sRNAs that specifically bind the Hfq protein (e.g. DsrA, MicF, RyhB, SgrS and RydC) control the translation of selected mRNAs in response to environmental stress (Gottesman, 2004). Structural and biochemical data indicate that Hfq folds into the shape of a hexameric ring in both *St. aureus* (Schumacher *et al.*, 2002) and *E. coli* (Sauter *et al.*, 2003). Hfq binds single-stranded RNAs (Franze de Fernandez *et al.*, 1972), and more precisely the protein interacts with sRNAs and mRNAs at A/U-rich single-strand sequences followed by an RNA helix (Brescia *et al.*, 2003). A subset of the sRNAs that bind Hfq, such as Spot42 or DsrA, harbor a 5'-AUUUUUG-3' sequence abutting a stem-loop, the consensual 'Hfq recognition motif' (Moller *et al.*, 2002; Brescia *et al.*, 2003), as is the case for the eukaryotic Sm (Hfq-like) proteins (Schumacher *et al.*, 2002). Recent work suggests that *E. coli* Hfq has two distinct RNA-binding surfaces and that two Hfq hexamers bind one sRNA (Sun & Wartell, 2006).

In *E. coli*, Hfq interacts with at least 40% of the known sRNAs and probably with additional ones (Wassarman *et al.*, 2001). Past and recent (Arluison *et al.*, 2007) data on the ability of Hfq to bind sRNAs and mRNAs suggest that the protein is an RNA chaperone (Moll *et al.*, 2003; Gottesman, 2004). The hexameric protein partially unfolds the RNA domains that interact during sRNA–mRNA regula-

tion, facilitating the initial recognition between the regulator and its target(s) or increasing the local concentration of the two RNAs. After binding, Hfq probably leaves the RNA duplex, and additional pairing between the two RNAs occurs, strengthening the sRNA–mRNA duplex. This interaction can result in the sequestration or exposure of the Shine–Dalgarno sequence from the mRNA targets or the initiation of mRNA degradation by RNase E (Afonyushkin *et al.*, 2005) or RNase III (Huntzinger *et al.*, 2005). In *E. coli*, 20% of the Hfq proteins copurify with RNase E, but how the protein targets the mRNAs for degradation or stabilization is unknown (Morita *et al.*, 2005). In *Listeria monocytogenes* (Mandin *et al.*, 2007) and *Vibrio cholerae* (Ding *et al.*, 2004), the expression of some virulence genes depends on the presence of the Hfq protein. In *Borrelia burgdorferi*, genes encoding Hfq and RNase E have not been identified, and this bacterium expresses very low number of antisense sRNAs (Ostberg *et al.*, 2004), suggesting coevolution of these two proteins with their RNA ligands.

Protein metabolism

As some bacterial small RNAs regulate gene expression at the translational level, it was expected that proteins involved in protein metabolism may interact with them, forming functional ribonucleoprotein complexes. These sRNA-binding proteins can perform catalysis on the sRNA substrates, which are used as specific transporters to direct them to the translational machinery. Some proteins are involved in the sRNA-mediated translational regulation; others are directly involved in the translation of the sRNAs that contain translatable internal ORFs. Other proteins use the sRNA as a scaffold to either stabilize a functional conformation or to enhance complex association/dissociation with a second protein. All these proteins are involved in the readout of the genetic information as well as in the targeting of signal-bearing proteins to the prokaryotic plasma membrane (Fig. 3).

Translation

Several bacterial regulatory RNAs interact with protein components of the translational apparatus (Fig. 3). Aminoacyl-tRNA synthetases (AARSs) are responsible for genetic code fidelity in ensuring that the correct amino acid is loaded onto the 3'-end of the corresponding tRNA. Non-canonical substrates of AARSs include the genomic RNA 3'-ends of several genera of plant viruses (Fechter *et al.*, 2001) and one sRNA, tmRNA, involved in ribosome rescue and in targeting aberrant proteins to degradation (Saguy *et al.*, 2005). The tRNA portion of tmRNA can be recognized and aminoacylated by the alanyl-tRNA synthetase, because it contains a key identity determinant (a GU pair) at the third position in the acceptor stem, as for tRNAs. Therefore,

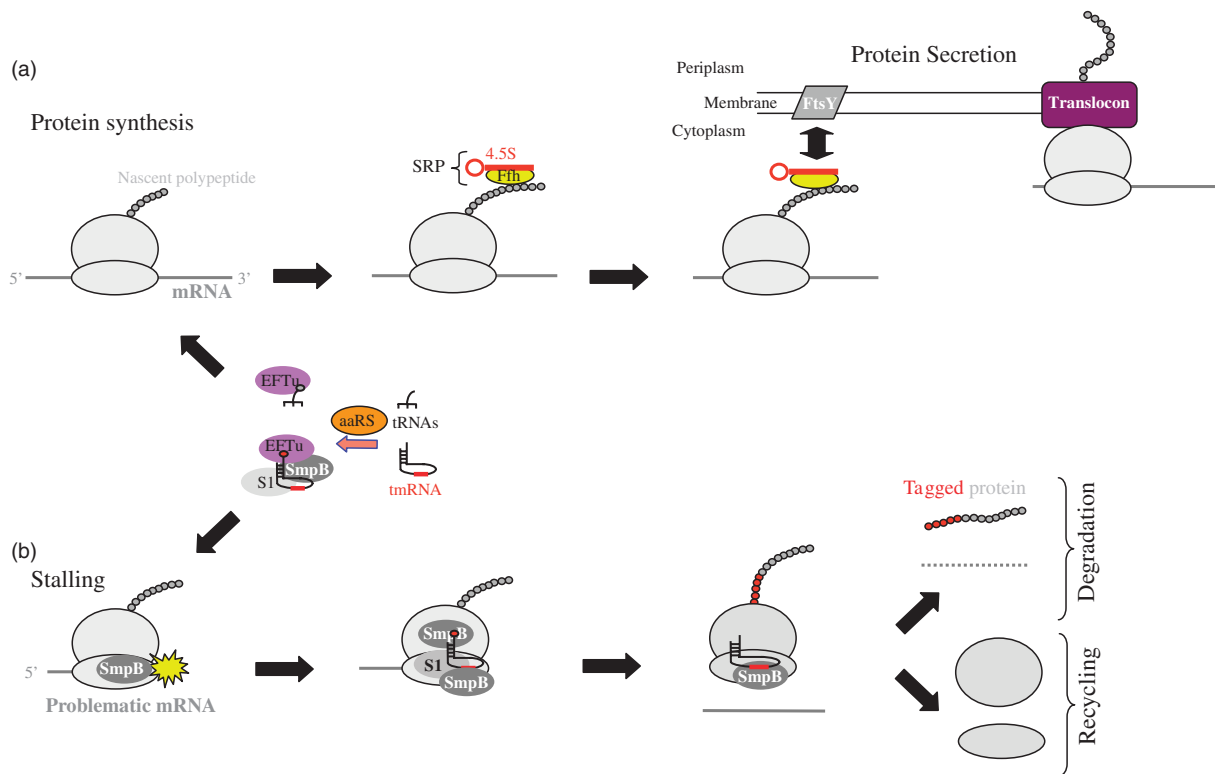


Fig. 3. The proteins that interact with bacterial sRNAs during protein metabolism. During translation and *trans*-translation, tRNAs and tmRNA both interact with aminoacyl-tRNA synthetases (aaRSs, orange; the alanyl-tRNA synthetase for tmRNA), and these aminoacylated RNAs are brought to the translational machinery via EF-Tu (purple). (a) For the secretory proteins, a nascent polypeptide chain (green) that is actively translated by the ribosome (the two gray subunits) contains an N-terminal signal sequence that is bound by the SRP (the 4.5S sRNA is red, and the Ffh protein is yellow), which arrests translation. The SRP-ribosome complex is targeted at the translocation complex embedded in the bacterial membrane via an interaction with the membrane-bound FtsY receptor (green). Following the docking of the ribosome with the translocon (purple), the signal sequence is released from the SRP, and the SRP is released from the SRP receptor in a GTP-dependent fashion. (b) When a ribosome stalls on a problematic message (yellow star), alanyl-tmRNA^{Ala} recognizes ribosomes stalled at the 3'-end of an mRNA fragment and adds the alanine to the C-terminus of the nascent polypeptide chain. Following mRNA swapping, the tmRNA ORF (red) is translated, and RF1/RF2-mediated termination releases the tagged protein (red-green) for degradation by cellular proteases and the problematic message for degradation by RNase R (Richards *et al.*, 2006), thus liberating the 30S and 50S subunits from the previously stalled ribosome for new rounds of protein synthesis. During the process, SmpB binds the stalled ribosome in association with tmRNA or prior to recruiting tmRNA. During preaccommodation, SmpB is still bound to the large and the small ribosomal subunits. The accommodation results in the movement of both the alanyl-tmRNA^{Ala} and SmpB bound to the 50S subunit. Subsequently, there is a codon-independent transfer of the peptidyl residue stalled in the P-site to the alanine from tmRNA. The molecule of SmpB bound to the 30S subunit either binds tmRNA at different sites from the tRNA-like domain or is released from the ribosome (Hallier *et al.*, 2006). The presence of ribosomal protein S1 (light gray) influences the position and structure of the tmRNA ORF, implying that S1 is an important factor for sending the ORF into the decoding site (Gillet *et al.*, 2007; Saguy *et al.*, 2007).

a bacterial AARS is able to aminoacylate an sRNA. The delivery of aminoacyl-tRNAs, the primary substrates of the ribosome, relies on the formation of a ternary complex with elongation factor Tu (EF-Tu) and GTP. Alanyl-tmRNA is also delivered to the stalled ribosome by EF-Tu (Rudinger-Thirion *et al.*, 1999). Proteins involved in translation, including EF-Tu, ribosomal protein S1 and probably others, are able to interact with some regulatory RNAs that contain (tmRNA) or do not carry (DsrA) translatable internal ORFs (see below for references). The question as to whether they contact the same ribosomal proteins during translation as canonical mRNAs do remains open, especially for those

containing intricate secondary structures, as is the case with RNAIII (Benito *et al.*, 2000) and tmRNA (Felden *et al.*, 1997). Of interest is ribosomal protein S1, which interacts with some regulatory RNAs, such as tmRNA (Wower *et al.*, 2000), DsrA (Koleva *et al.*, 2006) and possibly others (Schlax *et al.*, unpublished results). On the basis of cryo-electron microscopy (EM) (Gillet *et al.*, 2007) and *in vivo* data (Saguy *et al.*, 2007), S1 induces a functional conformation of tmRNA capable of being translated by the stalled ribosomes (tmRNA has an internal coding sequence). It allows translation reinitiation and peptide tagging of the problematic protein for protease degradation. Interestingly, S1 and Hfq

copurify with the bacterial RNA polymerase (Sukhodolets & Garges, 2003). As S1 has a critical role in translation initiation, this ternary interaction is probably important for 'transcription-translation' coupling. SmpB is a universal cofactor of tmRNA (Karzai *et al.*, 1999), adopts a β -barrel fold in solution and binds the tRNA part of tmRNA in the elbow region (Fig. 1c) on the D-like loop face (Gutmann *et al.*, 2003). The protein has additional binding sites, including one around the first codon of the tmRNA reading frame, where translation resumes (Metzinger *et al.*, 2005). SmpB interacts with the large and the small subunits of the stalled ribosome, and the affinity of the protein for the two ribosomal subunits is modulated by tmRNA in the course of *trans*-translation (Hallier *et al.*, 2006). Strikingly, two copies of the same protein interact with two different functional sites of the ribosomes.

Protein trafficking

The signal recognition particle (SRP) is a ribonucleoprotein that is essential for the targeting of signal peptide-bearing proteins to the prokaryotic plasma membrane (Doudna & Batey, 2004). SRP binds to the signal peptide emerging from the exit site of the ribosome and forms a ribosome nascent chain-SRP complex. The complex docks in a GTP-dependent manner with a membrane anchored SRP receptor (the bacterial SRP receptor is FtsY), and the protein is translocated across or integrated into the membrane through a channel called the translocon (Fig. 3). *Escherichia coli* SRP is the simplest known SRP, consisting of 4.5S RNA and one protein, referred to as p48 or 54 homolog (Ffh). The crystal structure of an *E. coli* '4.5S RNA-Ffh core domain' complex (Batey *et al.*, 2000) revealed contacts between two internal loops in the RNA and a series of conserved amino acids in the core domain (Fig. 1d). The 4.5S RNA stabilizes the structure of the Ffh core domain, and the RNA enhances both association and dissociation of the complex.

Quality controls

RNA folding

To accomplish their functions, bacterial sRNAs fold into accurate secondary and tertiary structures. During transcription, the RNA starts to fold and can fall into kinetic traps that lead to inactive misfolded RNAs. In *Gammaproteobacteria* and *Deinococcus*, the two proteins StpA (Zhang & Belfort, 1992) and Rsr (Ro 60 related, Chen *et al.*, 2000), respectively, are involved in 'RNA quality-control' mechanisms, but much has to be learned on how these systems work at the molecular level.

StpA is one of the most abundant proteins from the *E. coli* nucleoid. StpA regulates gene expression at transcriptional and posttranscriptional levels (Deighan *et al.*, 2000;

Delihias & Forst, 2001), including the gene expressing the outer membrane porin F (*ompF*). The sRNA MicF inhibits *ompF* translation by pairing with the 5'-UTR of the mRNA, near the Shine-Dalgarno sequence (Zhang & Belfort, 1992). StpA destabilizes MicF, and promotes its decay, probably by interfering with its structure (Deighan *et al.*, 2000). Recent work suggests that StpA binds misfolded RNAs to prevent kinetic traps, promoting RNA tertiary structure disorganization, followed by reorganization (Mayer *et al.*, 2007).

Another example has been observed for the 'radiation-resistant' bacterium *Deinococcus radiodurans*. Under high UV exposure, the Rsr protein and four sRNAs recycle misfolded RNAs (Chen *et al.*, 2000). Both Rsr and the sRNAs have sequence and functional homologies with the eukaryotic Ro protein and the Y sRNAs, respectively (Chen *et al.*, 2000). On the basis of the atomic structure of the eukaryotic complex (Stein *et al.*, 2005), the protein binds the 3'-end of the misfolded RNAs that unfold inside the 'doughnut-shaped' protein prior to recovering their functional structures.

Protein quality control

A eubacterial ribosome stalled on a defective mRNA can be released through a quality-control mechanism referred to as *trans*-translation, which depends on the coordinated binding actions of a unique chimeric sRNA acting as both tRNA and mRNA (tmRNA), SmpB and ribosome protein S1. This process leads to the release of both the tagged polypeptide to be degraded by dedicated bacterial proteases and the stalled ribosome to be recycled. Recent structural data obtained by means of cryo-EM suggest the unifying concept of scaffolding for the roles of SmpB and S1 in binding of tmRNA to the stalled ribosome during *trans*-translation (Gillet *et al.*, 2007).

Global regulators

Carbon storage regulatory proteins

The CsrA protein, a carbon storage regulator (Csr), binds RNAs. CsrA regulates carbon source utilization, glycogen synthesis, biofilm formation and motility in *E. coli* in regulating translation initiation of target mRNAs (Romeo, 1998). There are CsrA homologs in *Erwinia carotovora* [RsmA, repressor of secondary metabolism (Cui *et al.*, 1999)] and in *Pseudomonas fluorescens* [RsmA and RsmE (Reimann *et al.*, 2005)] controlling the expression of various extracellular proteins, including virulence factors. Csr also regulates epithelial cell invasion by *Salmonella enterica* (Fortune *et al.*, 2006) and affects swarming of *Proteus mirabilis* (Liaw *et al.*, 2003) and of *Legionella pneumophila* (Fettes *et al.*, 2001). In *V. cholerae*, CsrA and three redundant sRNAs regulate quorum sensing, a process

allowing community-wide synchronization of gene expression (Lenz *et al.*, 2005).

Each of the known target mRNA transcripts contains multiple Csr and Rsm protein-binding sites. CsrA is a 61 amino acid dimeric protein that binds specific sequences in the 5'-UTRs of target mRNAs, altering message stability and/or translation and reducing ribosome binding. On the basis of CsrA NMR structure (Gutierrez *et al.*, 2005), one protein from the dimer recognizes a GGA sequence from an RNA hairpin at the 5'-UTR of target mRNAs next to the Shine-Dalgarno sequence, inducing a conformational change of the mRNA-CsrA complex that increases the affinity of the second molecule of the CsrA dimer for the downstream Shine-Dalgarno sequence, preventing mRNA translation and destabilizing the mRNA structure.

In these bacteria, up to three sRNAs, acting redundantly, control the activity of Csr and Rsm regulatory proteins. Multiple copies of these Csr and Rsm proteins are trapped by regulatory RNAs, such as CsrB-C (*E. coli*), RsmB (*Erwinia carotova*) or Rsm X-Z (*Ps. fluorescens*), in turn allowing translation of the mRNAs that are repressed by CsrA, RsmA and RsmE. CsrA exists in equilibrium between CsrB-C and CsrA-regulated mRNAs, implying that CsrB-C levels are a key determinant of CsrA activity in the cell. These noncoding RNAs bind to and sequester the CsrA-RsmA proteins, preventing them from interacting with mRNA targets, and antagonizing their activities. These regulatory RNAs possess multiple imperfect repeated sequences that are the protein-binding sites. High levels of the regulatory RNAs compete for the binding of CsrA-RsmA to their mRNA targets, derepressing mRNA translation inhibition.

Concluding remarks

Bacterial sRNAs have to fold into specific conformations to be functional. After synthesis, the folding of the primary sRNA transcript depends on the action of dedicated protein enzymes, RNA chaperones and RNA scaffolding proteins to ensure RNA integrity, folding and activity. This RNA quality-control machinery includes proteins that are usually restricted to a specific sRNA (Table 1). At least two proteins have RNA quality controls functioning for several sRNAs (Chen *et al.*, 2000; Deighan *et al.*, 2000). We predict that 'RNA quality control' in bacteria will be an exciting and very active field in the years to come, involving the characterization of novel proteins involved in this process. This review has focused on the proteins that interact with bacterial sRNA regulators, describing their involvement in sRNA production, maturation, physiology and decay (Table 1). In *E. coli*, at least 90 sRNAs are expressed, and the number of proteins interacting with these sRNAs is currently unknown. In pursuit of this goal, only a few proteins involved in sRNA production and function have been identified so far. Several

proteins have been reported to interact with sRNAs, but in most cases the functional implications of this are poorly understood. As an example, a 60–70-nucleotide sRNA copurifies with a protein member of the blue-light photoreceptor in *V. cholerae* (Worthington *et al.*, 2003). The current challenge in this exciting field is to elucidate the role of these sRNA-protein complexes in bacterial physiology. We can predict that the identification of functional sRNA-protein complexes in bacteria will increase in the years to come, especially with the wealth of sRNAs recently identified in many bacteria, some of which have to interact with proteins for their function.

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